Piggybac-mediated stable expression of NGN2 in iPSCs for differentiation into excitatory glutamatergic neurons

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ABSTRACT

We adapted a previously-described method (Pantazis et al., 2022) for employing Piggybac transfection to stably express doxycycline-inducible NGN2 in human iPSCs. After stable integration of NGN2, proceed to differentiate iPSCs using protocol “iNeuron differentiation from human iPSCs.”

ATTACHMENTS

549-1145.pdf

GUIDELINES

Citations:

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Protocol status: Working
We use this protocol and it's working.

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PROTOCOL integer ID: 71238

Keywords: iPSC, Differentiation, iNeuron, Piggybac, NGN2

MATERIALS

Materials

- 10 cm cell culture dish
- 6-well cell culture dish
- Cryovials

Reagents

- Growth Factor Reduced (GFR) Matrigel® Corning Catalog #354230
- Essential 8™ Medium Gibco, ThermoFisher Catalog #A1517001
- Accutase® solution Sigma Aldrich Catalog #A6964
- Y-27632 2HCl Selleckchem Catalog #S1049
- Opti-MEM™ I Reduced Serum Medium Thermo Fisher Catalog #31985070
- Lipofectamine™ Stem Transfection Reagent Thermo Fisher Scientific Catalog #STEM00008
- PB-TO-hNGN2 addgene Catalog #172115
- piggyBac™ transposase vector (Transposagen)
- KnockOut™ Serum Replacement Thermo Fisher Catalog #10828010
- DMSO (CATALOG)

SAFETY WARNINGS

- Wear proper PPE when transferring cryovials to liquid N2.

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Oct 23 2023
1. Culture iPSCs in a 10 cm dish coated with Growth Factor Reduced Matrigel (Corning) and feed daily with Essential 8 media (ThermoFisher).

2. Passage iPSCs with warm Accutase into Essential 8 media with 10 micromolar (µM) ROCK inhibitor. Plate 800,000 iPSCs into one Matrigel-coated well of a 6-well plate.

3. 3 - 6 hours after plating, cells should be healthy and attached. Perform transfection using Lipofectamine Stem and a 2:1 ratio of donor plasmid to transposase:

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<tr>
<td>A</td>
<td>B</td>
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<tr>
<td>OptiMEM</td>
<td>200 µL</td>
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<tr>
<td>PB-T0-hNGN2-puro-BFP plasmid</td>
<td>0.75 µg</td>
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<tr>
<td>EF1α-transposase plasmid</td>
<td>0.37 µg</td>
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<tr>
<td>Lipofectamine Stem</td>
<td>4 µL</td>
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4. Check for transfection efficiency (BFP-labeled cells) on the next day using fluorescence microscopy.

4.1. Passage iPSCs with Accutase to a 10 cm dish when cells are confluent enough for splitting.

Note: Continue to feed iPSCs daily with Essential 8 media without ROCK inhibitor, and confirm division of stably-expressing transfected cells (should observe local clusters of BFP-fluorescent cells).

5. 72:00:00 after transfection, select for transfected iPSCs with 0.5 µg/ml puromycin.
5.1 Confirm purity of surviving transfected cells with fluorescence microscopy. When population is pure, withdraw puromycin.

6 Cryopreserve selected iPSCs with

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<tbody>
<tr>
<td>Essential 8 media</td>
<td>70%</td>
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<tr>
<td>Knockout serum replacement</td>
<td>20%</td>
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<tr>
<td>DMSO</td>
<td>10%</td>
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<td>ROCK inhibitor (Supplement)</td>
<td>10 µM</td>
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6.1 Proceed to culture and induction to neuronal fate using doxycycline (see “Protocol: iNeuron differentiation from human iPSCs”).